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XXIV.—*Contributions to the Natural History of the Infusoria.*
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[Plate III.]

THE author commences by noticing the rapid progress made, during the last ten years, in respect of our acquaintance with the structure of the Infusoria, and, in particular, the establishment of the fact of sexual reproduction among them. He had the advantage of pursuing his researches under the eye of Prof. Stein, and procured the subjects for them in the neighbourhood of Leipzig.

Stein and Balbiani have both shown that among the Infusoria there is an act of conjugation, and a sexual reproduction consequent thereupon. For instance, they proved that during the conjugation of two individuals, spermatozoa were developed in the substance of the nucleolus, and that in all probability these organisms penetrated the nucleus and gave rise to the formation of germinal corpuscles and embryos. These observations were made on the *Paramecium aurelia* and *P. Bursaria*; and Engelmann is able to confirm their general accuracy. In two conjugated individuals of *Paramecium Bursaria* he observed the nucleolus to be much enlarged, and to constitute two capsules, each filled with rod-like corpuscles; but, though he followed up his examination of such conjugated forms until their separation, and even subsequently, he failed to remark the development of embryonic corpuscles. In one instance, conjugation was extended to three individuals of *Paramecium Bursaria*, in two of which the nucleus was much extended and band-like, whilst in the third it retained its usual oval form. This triple conjugation he witnessed in only one other animalcule, viz. in *Vorticella convallaria.* Claparède records the fact in *V. microstoma.*

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* Abstract, by Dr. Arlidge, from the 'Zeitschrift für wissenschaftliche Zoologie,' vol. xi. part 4 (1861).

Engelmann confirms John Müller's discovery of spermatozoa in *Paramecium aurelia,* the nucleus being more or less enlarged and filled with them. When pressed out, they exhibit no independent power of motion. They are not, as usually represented, thin rods equally pointed at both extremities, but have a more bulky anterior and a thinner posterior extremity of greater transparency. Their maximum length is 0·008 of a millimètre. Among specimens of conjugated *Paramecia,* some, evidently only recently united, possessed a nucleus of the usual oval form; in others it was more or less spherical, and presented constrictions at different portions of its surface; whilst in others, again, it consisted of a coil of longer or shorter band-like segments. Among such examples of conjugated forms, he also met with single beings which contained, instead of a nucleus, from two to four larger oval or round bodies, and a number of similar but smaller ones. What had become of the nucleolus in these beings, Engelmann was unable to surmise, nor could he, notwithstanding his best efforts, discover the large seminal capsules recently described by Balbiani. Lastly, individuals were met with having embryonic globules, mostly lying in the posterior half of the animals; and frequently acinetiform embryos were seen to detach themselves from these globules and make their way to the surface through a special wide canal in the parent animalcule.

Engelmann observed conjugation, besides, in *Paramecium ambiguum,* a new species, found in the lake at Eisleben, having the figure of *P. Bursaria,* but colourless like *P. aurelia,* and having a longer bundle of cilia at the posterior extremity.

Instances of conjugation were also encountered in *Paramecium Colpoda* (*Colpidium,* Stein), in several species of *Trichoda, Cyclidium glaucoma, Cinetochilum margaritaceum, Coleps hirtus,* in a small *Prorodon,* in *Nassula aurea, Lacrymaria elegans* (new species), and in *Amphileptus fasciola.* With the exception of the conjugated individuals of the species last named, the union took place at the fore part of the body; so that where the mouth occupied that position, as in *Coleps, Prorodon,* and *Lacrymaria,* the oral apertures were in apposition.

In the case of *Amphileptus,* the animals were united throughout their length, with the exception of the distal ends of their long necks and the contracted posterior extremities. No more advanced phases of development were met with among the conjugated individuals; but, in company with those of *Amphileptus fasciola,* unusually broad specimens were encountered at times, which possessed four nuclear bodies instead of the usual two spherical nuclei. In such instances Engelmann surmises that the act of conjugation has occurred; and he also finds parallel examples in Oxytrichina. The process of conjugation he further noticed partially on one occasion in *Vorticella Convallaria,* in *V. microstoma,* and in *Urocentrum Turbo.*

Stein has figured *Chilodon cucullulus* in conjugation, and Engelmann has witnessed several stages of the process. Among specimens of this animalcule, he met with some having the dorsum of one individual united with the ventral surface of another, and, again, other specimens with the ventral surfaces in apposition and the oral openings closely conjoined. He confirms the existence of rod-like corpuscles in the nucleus of this species, and remarks on some examples where the nucleus had become spherical and contained such corpuscles scattered irregularly within it, and resembling in figure those of *Paramecium aurelia,* but only one-half their length.

Stein observed conjugation in *Aspidisca lynceus* and *A. costata*; to these Engelmann adds *A. turrita,* and states that he has met with examples where the right border of the ventral aspect of one animalcule has overlain the left border of the dorsum of the other. He questions whether this position represents a later stage of development, since, at the first, the left side of the right animalcule is opposed to the right border of the dorsum of the left animalcule, as is seen likewise in Oxytrichina (Pl. III. figs. 4–6). The nucleolus of Aspidiscina, previously overlooked by observers, is stated by Engelmann to be of comparatively large size, and always placed, as in *Euplotes,* more or less close to the middle of the left side of the body, and in intimate contiguity with the horseshoe-shaped nucleus. This last sometimes presents, at no great distance from each of its two extremities, a transverse fissure-like cavity, such as Stein has figured in *Euplotes* and Oxytrichina.

Conjugation was noticed by Ehrenberg in *Euplotes,* but mistaken by him for longitudinal fission. Stein and Balbiani have since studied the process in this genus, arriving at the conclusion that it is an act of sexual reproduction. Commonly the two individuals are united along the left of their ventral aspect (fig. 1); the nucleus is horseshoe-shaped, the nucleolus distinct from it, and eventually divided into two progressively receding segments. The conjugation is limited to the portion of the body anterior to the peristome. Among such conjugated ordinary specimens are others which present a newly-formed adoral fringe of cilia on the posterior half of the ventral surface, and an entirely new system of ventral cilia (fig. 2). These new cilia progressively enlarge in length and width, and become closely set, whilst the old cilia so far disappear that, when conjugation is at an end, only 3–4 of the anterior and a few of the posterior are extant. At the same time two curved longitudinal folds make their appearance, extending backwards or inwards from the newly formed wreath of adoral cilia, constituting the boundary of a new peristome, which proceeds to develope as the old one progressively vanishes along with its surrounding cilia (Pl. III. figs. 2, 3). Even after the separation of two of these peculiarly conjugated animalcules, many of the characteristics of the usual forms of *Euplotes Charon* are deficient, one of the most remarkable of which is the absence of a mouth; for the adoral cilia end at the middle of the area of the peristome, and the two folds indicating this structure coalesce so as to constitute a rib-like elevation ending at the base of the fifth posterior cilium. Moreover the ventral surface is traversed by a distinct fold, not seen or very slightly developed in animals of the ordinary type. The most striking variation, however, in the new being is witnessed in the nucleus, which divides into two portions either before or simultaneously with the commencement of the formation of the new ciliary appendages in the conjugated animals. One segment is anterior, and much larger than the other, and occupies the whole anterior third of the ventral cavity, whilst the other, globular in figure, is thrust backwards and to the left side. The nucleolus undergoes fission yet earlier, and one section of it continues in apposition with each portion of the nucleus. After the separation of the animals a pale discoid body is noticed near the posterior extremity and on the left side, which gradually advances more forward and to the centre, and acquires an increased diameter and transparency, until at length it forms a perfectly clear, somewhat compressed spherical body, filling ofttimes two-thirds of the whole interior of the animalcule, and occupied by an extremely fine, granular, homogeneous substance (fig. 3). Beside or behind this body, a considerable portion of the original nucleus is noticeable, together with a constantly fluctuating number of larger and smaller, strongly refracting (fat?) corpuscles. Two or three days, or thereabouts, after the separation of the two individuals, they are found to have re-assumed the customary form and structure of the species, including the peristome, mouth, and the original horseshoe-shaped nucleus.

The question suggests itself, What is the origin of the large transparent globule in the interior, and what purpose does it serve? To this question Engelmann is at present unable to give a decisive answer. He is in doubt whether this corpuscle originates from the posterior half of the nucleus after its fission, or whether it takes its rise prior to the detachment of the conjugated animals, as an independent formation from the nucleus. The latter assumption derives support from the circumstance that, in two conjugated individuals on the point of separating, there existed, besides the two detached halves of the nucleus, a clear, delicate, and sharply defined globular body in the posterior half of each animal. Nevertheless Engelmann did not see this structure in a large number of other specimens at the same stage of conjugation.

In the course of the further development of the bodies in question, they break up, after attaining their maximum distention, into several (mostly from two to three) spherical segments of nearly equal or of different dimensions. The further history of these corpuscles is unknown, though the idea may be hazarded that they are concerned in the formation of germinal corpuscles. But whether these last, when formed, are further developed into embryos within the parent beings is questionable; on the other hand, Engelmann traced the re-assumption by a conjugated pair of the ordinary characters of *Euplotes Charon* without seeing any embryonic development take place. But, further, it is possible that the germinal corpuscles of *Euplotes* may not, as in Vorticellina, attain their maturity within the parent, but may do so externally to it. In this opinion Balbiani seems to concur.

Engelmann has observed two entirely different forms of conjugation in *Stylonychia pustulata* and in *S. histrio*. In one variety, two originally independent individuals so completely coalesce that in the end the resultant being is undistinguishable from any ordinary example of the species. In the other form, the conjugation is limited in its results to the formation of a new ciliary apparatus on the anterior portions of the conjoined animals, which gradually replaces the old one; after this is accomplished, separation takes place. This latter form has been already noticed in the case of *Euplotes*.

The act of conjugation in *Stylonychia pustulata* (Pl. III. fig. 4) commences by the adhesion of the two animals by their adoral wreaths and strong frontal uncini, so that the left anterior extremity of one is overlapped by the right of the other. Soon a band of union, composed of the substance of the body, unites the two, and gradually increases in width, the bristles and uncini, together with the adoral cilia, disappearing in the meantime in the case of the left individual. Within a short time, the bodies of the two beings have so coalesced that they have an anterior extremity in common, and a divided body posteriorly; but even this latter indication of their original duality gradually vanishes, until at length the two have become as one being, undistinguishable from an ordinary species of *Stylonychia pustulata*, except perhaps that it is commonly broader. In this process the four nuclei have also coalesced into two. This act of complete coalescence is accomplished in from twelve to fourteen hours. On watching the subsequent history of the compound being, it is found to commence division after from six to ten hours' union; and this unloosing is at length completed, the resultant individuals differing in no perceptible features from the ordinary specimens of the species; and, as far as Engelmann could make out, all this marvellous process stood in no relation whatever with sexual reproduction.

The second variety of conjugation has already been described by Stein in *Stylonychia pustulata* and *S. histrio*. In a not uncommon form of this partial conjugation, the two individuals are found united only by their anterior extremities, and a reproduction of the ciliary apparatus of the ventral surface is seen to proceed (Pl. III. fig. 5). This continues to extend itself, and the adoral wreath of cilia especially stretches forward, whilst the old adoral and frontal cilia become absorbed (fig. 6). The act of fission extends from behind forwards, until the separation is complete (fig. 7). All this is effected in such a way that the individual on the right side retains the residue of the old frontal region with its three largest bristles and the remnants of the old adoral ciliary wreath; but these original parts themselves vanish in another fifteen minutes. The nucleus has in the meantime undergone various changes, and generally appears broken up into several spherical segments. The beings resulting from these metamorphoses resemble those of *Euplotes Charon* in being destitute of a mouth and peristome, and in giving origin to those peculiar corpuscles, having a large transparent central body, which are always found in company with the conjugated forms of *Stylonychia pustulata*. Engelmann followed up the observation of some such specimens, and found that, after a while, they resumed the characters of the species, and proceeded to undergo fission, or else, as in the case of some, they underwent the peculiar process of encysting.

Stylonychia histrio exhibits, in all essential particulars, the same two forms of conjugation.

Once, among various examples of *Stylonychia histrio* in conjugation, Engelmann met with an animalcule of the same species, which exhibited, in the place of the nucleus, three large, clear, discoid bodies, of which one possessed a central granular and circular nucleus (Pl. III. fig. 8). After rather more than an hour, two of these bodies began to coalesce, so that, three hours afterwards, the interior of the animal presented one much larger discoid body, and behind this a smaller one; but, twenty minutes later, this last also first adhered and then ultimately united with the other, so that in the end the individual presented a single large spherical body having a granular central nucleus. The further history of this animalcule could not be traced, as it was lost to observation by death.

To Stein's account of the conjugation of *Stylonychia Mytilus* Engelmann adds that in one instance two individuals were coherent by their posterior extremities, and that the two varieties of conjugation noticed in the other species above described occur likewise in this one.

The embryonic development observed by Stein also fell under the notice of Engelmann. In 1859 he found specimens containing embryonic corpuscles; but it was not until the autumn of 1861 that he met with examples which illustrated a further phase in their history. These latter were individuals of medium size, and mostly contained but one large embryonic globule, placed between the two nuclei, close behind the angle of the oral aperture. Placed over it, on the ventral aspect of the animal, there always existed an elliptic or rounded opening, of variable size, which was the outlet for the escape of the mature ovum. On one occasion only was an elongated and rounded dorsal aperture found, in addition to the abdominal foramen just named, and serving, like it, for the escape of the embryos. The act of birth was several times witnessed: sometimes the embryonic globules escaped as such, at others they developed tentacles and assumed the acinetiform figure as usually described. The subsequent history of the embryos is unknown to Engelmann; however, he does not believe in their immediate transition to the ordinary form of *Stylonychia*; for the smallest examples of the species are more than ten times the length of the largest embryos, and he is therefore disposed to accede to Stein's view, that an "alternation of generation" takes place.

Engelmann protests strongly against the doctrine recently promulgated by Balbiani, that the acinetiform beings seen to emerge from the interior of various Infusoria, and generally held to be their embryos, are nothing less than parasitic Acinetæ (*Sphærophrya* of Claparède), which have previously made their way into them. If this were the case, it would be difficult to explain why the Acineta should always penetrate *Stylonychia Mytilus*, for example, at the same spot; for the oviduct is constant in position, and the integument of the animalcule of considerable firmness, particularly at the part perforated. Balbiani indeed figures a *Stylonychia Mytilus* with three dorsal apertures; but such a specimen Engelmann has never encountered, in more than a hundred individuals examined by him containing embryonic corpuscles. Is it likely that a parasite should always penetrate at one spot in *Stylonychia Mytilus*, whilst in other Infusoria, as, for example, in *Paramecium aurelia* and *Urostyla grandis*, which have several oviducts, it should pierce at several points? Why, again, should the supposed parasite bore its way into some species, whilst in others, even those closely allied (as

Stylonychia histrio), it should never be known to enter? It is moreover very doubtful if such beings as the acinetiform embryos of *Stylonychia* or of *Paramecium* are so organized as to be able to penetrate their bodies from without. Their structure, indeed, teaches to the contrary; for their soft, weak tentacula are not calculated to penetrate the resistant cuticle of *Stylonychia* or the thick wall of *Paramecium* with its contained acicular bodies (trichocysts or thread-cells of Allman), and to hollow out a wide canal such as we meet with in those Infusoria. On the other hand, there are direct observations opposed to this hypothesis. Thus, Engelmann recounts several instances in which small Acinetæ have affixed themselves to Infusoria and withdrawn their contents by the agency of their tentacula, but have in no degree bored through their integuments. Again, the uniformity always met with in the structure and contents of the embryonic globules is remarkable. In these particulars they exactly resemble those of Vorticellina, the non-parasitic character of which is unquestioned. On the contrary, the substance of the smallest Acinetæ is dissimilar; for this, instead of being clear, homogeneous, and destitute of granules, is highly granular, cloudy, and occupied or discoloured with absorbed nutritive material. Lastly, the circumstance is to be noted that the animalcules with which such acinetiform beings are connected, are actually at the time concerned in the development of germs; and the rule is, as Stein pointed out, that acinetiform embryos are found in most or in all the examples of a species existing in the same fluid—a fact which also holds good with respect to the phenomenon of conjugation. If due weight be allowed to these various considerations, the opinion that the acinetiform beings in question are really extraneous parasitic Acinetæ cannot with propriety be maintained.

Stein was the first to collect facts relative to the development of the embryonic corpuscles of *Stylonychia Mytilus*, by showing the conditions and changes of the nuclei and of the nucleoli. Among numerous examples of individual animalcules containing several embryonic corpuscles, only a few presented the two normal nuclei, with either no nucleolus at all, or a single one. In by far the greater number, the nuclei were replaced by three or four, rarely six, oval or sometimes round bodies, and from two to four nucleoli of larger size than in the ordinary specimens of the species (Pl. III. fig. 9). Sometimes three or four nuclear bodies were present but no nucleolus. The nature of two of these spherical or oval bodies having the widest dimensions is in all probability that of nuclei, whilst the rest may be considered embryonic corpuscles. These last do not seem always to originate in the same fashion: thus, in some cases, the nucleus is seen to break up into several segments, of which two retain the nuclear character, whilst the rest become perfectly homogeneous and exhibit no nuclei in their interior. In other examples a few nuclear-looking structures take their rise within the substance of the nucleus, at the enlarged end of the elongated and sometimes ramified nucleus. According to Engelmann's observations, the germinal corpuscle arises by a constriction of a portion of the enlarged nucleus, which proceeds to complete separation, and contains within itself a nuclear particle; for he has never been able to discover such a body lying within a special cavity of the nucleus. Moreover those spherical corpuscles which differ from germinal bodies only by the absence of nuclei within them, and which appear to originate by repeated fission of the original nucleus, eventually become germinal and embryonic corpuscles. Lastly, these embryonic bodies undergo repeated fission, and are ultimately transformed into embryos.

A very remarkable phenomenon was noted by Engelmann in very many specimens of *Stylonychia Mytilus* containing embryonic corpuscles, viz. that, in addition to these bodies, there were also present in them several (often as many as four) examples of the beautiful cysts of *Podophrya fixa*, which have been described by Weisse under the name of *Orcula*. Among other specimens of the same animalcule the same observer also met with some which, instead of two nuclei, possessed two large clear spherical bodies. In one of these animals, each globule presented a nucleus with an irregular outline, giving off from its periphery several delicate processes like those of *Amœbæ* and other Rhizopods. Three hours later, these nuclei had acquired a clustered outline, and had evidently broken up into a multitude of sharply defined, rounded particles, still retained in relation with the periphery of the enclosing corpuscle by fine filiform processes. Seven hours afterwards this specimen was unfortunately destroyed by the evaporation of the water around it.

In some other Oxytrichina, Engelmann finds similar conditions of conjugation as in the *Stylonychiæ* quoted, and among others in *Pleurotricha lanceolata*. Another phenomenon noticed by him in the animalcule just named is that of encysting. The resulting cysts are precisely like those of *Stylonychia Mytilus* in appearance, but of much greater size. Conjugation was also investigated by him in *Oxytricha pellionella*, in *O. ferruginea*, and in *O. parallela*—the last-named a new species.

To sum up the foregoing observations:—Two forms of conjugation occur in the class of Infusoria, but only one of these appears to be connected with the process of reproduction. The first form consists in the complete coalescence of two previously distinct individuals into one being. Though hitherto noticed only in some of the Oxytrichina, its occurrence is rendered probable, by Claparede's researches, in *Vorticella microstoma, Carchesium polypinum, Epistylis brevipes,* and in several Acinetina. All that can be predicated with certainty respecting this variety of conjugation is, that it has no direct relation to the process of reproduction, but appears analogous to the coalescence observed between individuals of the class of Rhizopods, particularly those of the genus *Actinophrys.*

The second form of conjugation proceeds by means of a union of a portion of the bodies only of two individuals, as a rule, of their anterior parts, in the region of the mouth; it persists several days, and then the animalcules once more separate. When separation has occurred, the two beings are found changed in various ways, both externally and internally, from their original characters, although, after the lapse of some days more, those primitive features are resumed. This statement holds good, at least, with respect to the Oxytrichina and Euplotina. But in the case of the *Paramecia* and *Colpidia,* and other holotrichous Infusoria, whose organization differs widely from that of the other two families named, the recently conjugated individuals differ very little or scarcely in any perceptible degree in external characters from the normal form. The purpose served by this second mode of conjugation, as particularly illustrated in the *Paramecia,* stands in immediate relation with the process of sexual reproduction. Another conclusion arrived at from the study of the conjugation process is, that in no species of Infusoria do longitudinal and transverse fission occur together, but every species multiplies either by one form or by the other alone.

It will not be uninteresting to append some observations respecting the nucleolus. Siebold seems to have been the first to notice this organ, in *Paramecium Bursaria.* The researches of Stein and Balbiani, however, have so added to the number of instances of its occurrence, that from thirty to forty may be enumerated. To these Engelmann is able to add a dozen other examples in other species. In *Glaucoma scintillans* the nucleolus occurs as a small round corpuscle lodged in a hemispherical cavity in the round nucleus. In *Frontaria leucas* three nucleoli of tolerable size may be brought into view, particularly after the application of water or acetic acid, adherent to the oval elongated nucleus. In *Trachelophyllum apiculatum* two comparatively large nucleoli are lodged, one in a hollow at each extremity of each of the two oval nuclei. *Conchophthirus anodontæ* (Pl. III. fig. 15) and *C. curtus* have one or two nucleoli lying close to or upon their oval nucleus. A nucleolus also occurs in *Aspidisca lynceus, A. turrita,* and *A. costata. Vorticella Convallaria* and *Epistylis flavicans* likewise possess a tolerably large nucleolus, usually situated on the concave side of their elongated nucleus. In several new forms described by Engelmann this same organ is found, viz., in *Drepanostoma striatum, Chasmatostoma reniforme, Lacrymaria elegans*; some new Oxytrichina, as *Pleurotrocha setifera, Gastrostyla Steinii, Uroleptus fragilis, Oxytricha strenua,* and *O. parallela.* With less certainty he refers to a nucleolus in *Opercularia coarctata, Zoothamnium Aselli, Urocentrum Turbo,* and in some other well-known Infusoria. On the other hand, he has convinced himself of its presence in all the Oxytrichina known to him, in *Euplotes,* in *Chilodon cucullulus, Nassula aurea, Prorodon teres, Lacrymaria olor, Panophrys flava* (=*Bursaria flava,* Ehr.), *Colpidium,* Stein (=*Paramecium colpoda*), *Balantidium entozoon, Paramecium Bursaria,* and *P. aurelia, Entodinium bursa* and *E. caudatum, Carchesium polypinum* and *Epistylis digitalis.* All Engelmann's attempts to find a nucleolus in *Epistylis plicatilis* have failed; and he is unable to confirm the existence of one in *Blepharisma lateritia,* although Balbiani has described it. Nevertheless in one specimen of the species last named he noticed a large sac, placed in front of the nucleus, filled with rigid, motionless, acicular bodies resembling, both before and after the addition of acetic acid, the spermatozoa of *Paramecium aurelia.* He found also other specimens of the same animalcule in which the nucleus was broken up into five or six spherical segments, of homogeneous consistence—an appearance Stein has also noted and figured.

In one and the same species, among several of the Infusoria, the nucleoli vary in number. A remarkable instance of this fact occurs in *Stylonychia Mytilus,* in which from two to four nucleoli are present. In *Urostyla Weissei* from two to eight occur; and *Stylonychia histrio* has at one time two, at another four nucleoli.

The purpose of the slit-like openings in the nucleus, as observed by Stein in Oxytrichina and in *Aspidisca,* is not determined. One supposition is, that they may serve an important end in sexual reproduction by giving passage to the spermatozoa. They are usually arranged quite symmetrically, and are approximated towards each other, or placed at opposite extremities of the nucleus. Anomalies, however, occur at times.

Engelmann remarks on a slit-like aperture in the area of the peristome of *Onychodromus,* lying obliquely across the direction of the anterior nucleus, and recalls, in connexion with this observation, the presence of a crescentic line described by Stein and Balbiani as dividing the peristome of *Stylonychia Mytilus* into an anterior and a posterior segment. Balbiani has further remarked the like structure in *Trachelius ovum,* and imagines that these slits may be structurally connected with the reproductive organs—an opinion Engelmann considers not improbable, although he is unable to adduce any facts in confirmation of it.

Engelmann, moreover, has been able to follow out the researches of Stein, Claparède, and Lachmann relative to the history of development in various hypotrichous and peritrichous Infusoria. In *Epistylis plicatilis* he met with those buds from the base, noticed by Stein, and called by Claparède *Urnulæ* (Pl. III. fig. 10), remarkable for their acinetiform development. They spring from the *Epistylis* at its junction with the pedicle, exhibit a rigid and more dwarfed outline than do the ordinary gemmæ, which, on the contrary, elongate themselves, and take on the ordinary characters of their parent. Engelmann witnessed the transverse division of these Urnulæ, but was unable to detect their further development in this species of *Epistylis*.

The same kind of organism is produced from the *Epistylis crassicollis*. The rounded, sharply defined body of the Urnula is enclosed in an oval, firm, and colourless sheath (Pl. III. fig. 10) having a tapering posterior extremity, and terminated anteriorly by a triangular aperture of moderate dimensions, through which the animalcule protrudes a single moveable tentacle, or, rarely, two such organs. In the interior of the animal a granular nucleus and one or more contractile vesicles are discernible. A transverse constriction followed by fission proceeds, and the anterior uniformly ciliated segment quits the sheath. The Urnula has a very close resemblance to *Acineta mystacina*, differing from it in no other respect than in the figure of its sheath and in its primitive position. Claparède assumed it to be a member of the family Rhizopoda, especially as he observed its tentacles ramify. This circumstance is not deemed by Engelmann of sufficient moment to induce its transfer to such a wholly different class of animals from that with which in all other respects it seems related. For, he asks, what Rhizopod has a sheath like *Urnula*, or what Rhizopods can employ their pseudopodia as sucking-tubules? or which of them, again, can multiply themselves by means of ciliated fission-products, as the Urnula can do? But in all these peculiarities the Urnula coincides with the true Acinetæ; whereas, if it were considered one of the Rhizopoda, it would be difficult to determine the boundary-line between this class and that of the Infusoria (Ciliata). The amphileptoid cysts, first described by Claparède and afterwards by Stein and Udekem in various Vorticellina, were seen by Engelmann also in *Epistylis plicatilis*. The *Amphileptus* underwent fission within the cyst before leaving it.

Similar Urnulæ and amphileptoid cysts were encountered in *Epistylis crassicollis*. In *E. flavicans*, which has a distinct oval nucleus, the preliminary stage in the formation of germinal corpuscles was observed taking place in the nucleus, and ending in the production either of a very large number of small ones closely packed and having a central vesicle, or else of a smaller number imbedded within the substance of the nucleus. At the same time, one, or, seldom, several elongated sharply outlined bodies, having clear vesicles internally, were visible in the nucleus; and these after a while broke up into many smaller segments. Within other nuclei one or more extended, sharply defined, homogeneous masses, of firmer consistence than the nuclei themselves, were detected, but no spherical corpuscles.

The somewhat reniform corpuscles remarked by Claparède scattered in pairs through the parenchyma of *Epistylis flavicans* appear to be peculiar to this animalcule. They strongly refract light, and vary much in number, sometimes are absent altogether. Their purpose is obscure.

In *Carchesium polypinum* Engelmann observed the origin of germs and embryonic corpuscles several times in May and October 1860. Usually only one globular germ appeared at a time, close under the integument of the anterior portion of the body, distinguishable by its clear homogeneous substance and its very large central nucleus. At one point in its periphery a hollow space, slowly changeable in its dimensions (a contractile vesicle), was also perceptible. The nucleus of the parent animal was always present unchanged in appearance.

In June 1860, Engelmann met with a specimen of *Carchesium polypinum* having a thick bud-like outgrowth from near its stem, gently tapering in front, with a circular opening at its apex. In its interior were several rather large, strongly refracting corpuscles, of an oval figure, not dissimilar to germinal corpuscles. In course of time this process collapsed, and hung like a flabby sac from the *Carchesium*. Stein has recorded a similar formation in *Vorticella nebulifera*.

But it was in the *Carchesium Aselli*, a new species discovered by himself, that Engelmann was enabled more fully to explore the phenomena of embryonic development. On the 1st of April, 1860, he met with a full-grown dead specimen of this species, containing a large spherical homogeneous body, 0·042 millimètre in diameter, possessing a round nucleus of considerable magnitude. Contiguous to it lay the smaller, curved, horseshoe-shaped nucleus of the animalcule. On the following day, he saw eight examples, each containing one such embryonic globule, which lay close beneath the peristome. A slowly contractile vesicle existed on the surface of each; and, by the addition of acetic acid, an opake distinctly defined nucleus was brought into view. The nucleus of the parent being could not in all cases be distinguished. In company with these forms were others, belonging evidently to a later phase of development. Thus the large circular nucleus of the embryonic body was subdivided into two oval segments, which, on the addition of acetic acid, were seen to be enclosed within the common transparent capsule of the embryonic corpuscle (Pl. III. fig. 12). One or two similar oval bodies are sometimes found in addition within the parenchyma of the animalcule; and occasionally the nucleus of the *Carchesium* is not discoverable. It also at times happens that an outgrowth or protuberance appears on the surface of the animalcule, either about its middle or at its anterior part; but these conical tapering swellings exhibit no distinct aperture, though possibly one may eventually make its appearance. In these prominences an embryonic corpuscle was several times seen; nevertheless it is doubtful whether the embryos make their exit through them, for Engelmann observed their escape through a fissure near the peristome (fig. 11). The escaped embryo is oval, very small, and furnished with two zones of long cilia in front, by means of which it swims about in an impetuous manner. Internally it presents a contractile space and a rounded nucleus (fig. 11*a*). From these observations Engelmann concludes it to be probable that one or several embryos are derived from the nucleus of the original embryonic corpuscle, which advance in growth at the expense of the matter contained within the corpuscle, and after a time effect their escape through its surrounding wall, and lastly through the coats of the parent animal by means of a special foramen. This interpretation coincides with the observations recorded by Claparède respecting the formation of embryos in the *Epistylis plicatilis*.

Engelmann has met with but few examples to illustrate the development of embryonic corpuscles from the nucleus of the parent animal. In one instance where there was a large embryonic body, the reduced nucleus presented several nuclear structures. In another animal which had no such large corpuscle, the nucleus was likewise reduced in size, and in contact with it were six small spherical corpuscles having a central vesicle. In a third example, along with a large embryonic body there were five rounded corpuscles, each with a central vesicle, apparently derived from a breaking-up of the nucleus, but differing materially in size among themselves. These were probably produced after the first embryonic corpuscle had developed its embryo, and a series of such corpuscles were prepared for successive elaboration into embryos.

In the production of embryonic corpuscles another phenomenon is presented in the case of *Carchesium Aselli*, corresponding with that seen by Stein in *Vorticella nebulifera*. This consists in the outgrowth of more or fewer large convex processes from the body of animals found in company with others bearing the embryonic corpuscles. These protuberances are mostly situated on the posterior half of the body, and contain a considerable number of small spherical refracting corpuscles, similar, indeed, to others collected in large numbers within the parenchyma of the parent animalcule. They are evidently the products of a fission-process in the nucleus, some very slight remnants of which are only now and then perceptible. In one instance these same corpuscles were noticed, though no outgrowths existed. The purpose of such products is still a question. Stein has supposed them to be connected with the origin of the fertilizing elements.

In *Carchesium Aselli* Engelmann further observed that peculiar condition in which the disk of the peristome assumes a spherical figure and projects from the strongly contracted animalcule (Pl. III. fig. 13) very much like a yet adherent embryo, but from which it is distinguished by its want of a nucleus and its absence of independent locomotion. If torn from the animal, it does not lose its cilia for some time, though it never swims far with them, but settles down to the bottom of the water, and soon lies motionless there.

The small corpuscles derived from a breaking-up of the nucleus have been seen also in *Zoothamnium affine*. The processes containing them are of very large size, and either of an elliptic elongated figure or conical and thick, surrounded by a short tubular-looking process, having a distinct aperture at its summit (fig. 14). The corpuscles themselves varied in size, but never exceeded 0·005 mill.

The extrusion of embryonic corpuscles was noticed in *Vorticella Convallaria*. Usually, as in *Carchesium polypinum*, only one such corpuscle, of considerable size, having an opake nucleus, was present.

The development of germinal corpuscles in *Didinium nasutum* (Stein) is interesting. This remarkable animalcule ordinarily presents a horseshoe-shaped nucleus, but no nucleolus; but in October 1860, Engelmann encountered very many specimens in which the nucleus enclosed numerous small spherical corpuscles having a central vesicle in their interior. Among these were several (four to six) considerably larger globular bodies, containing each a distinct nucleus. In other individuals the nucleus was seen divided into several more or less globular segments, lying close together, furnished severally with a nucleus, though still enclosed within the membrane of the parent nucleus. These structures assuredly represent the early phases in the formation of germinal and embryonic bodies. During the transverse fission of *Didinium*, the nucleus becomes much elongated, and then appears striped longitudinally; but on the completion of fission the longitudinal striæ disappear.

In the group of Acinetina, development by internally produced embryos has been demonstrated by Stein and Claparède in almost all the species known. Engelmann remarks that he has seen the embryos of *Acineta operculariæ, A. quadripartita, A. Astaci,* and *A. infusionum.* In the last-named species and in *A. quadripartita,* Engelmann further observed the reverting of the ciliated embryos to Acinetæ; and he remarks that the embryos of Acinetæ known to him are distinguished from those of ciliated Infusoria (e. g. *Stylonychia Mytilus, Paramecium aurelia, Epistylis plicatilis,* &c.) by the remarkable circumstance that their entire body is not, as in those of the latter animalcules, devoid of granules, and transparent, but filled with the same nutritive materials as occur in the parent beings. For example, the embryos of *Acineta quadripartita* and of *A. infusionum* are so filled with the fat-globules and other nutritive matters derived from the parent animals, that it is difficult to discover their nucleus and contractile sac. Indeed, this single circumstance renders it highly improbable that the entire Acineta-embryo can be derived solely from the substance of the parent nucleus. This last is certainly frequently seen broken up into numerous coarse granules, but only a small portion at most of the frequently extremely large and opake embryo can be derived from them; on the contrary, it is much more probable that only their nuclear portion arises from the nucleus of their parent, the rest of their large body being derived directly from the contents of the parent Acineta. Stein, in fact, has heretofore intimated the same. Thus in *Acineta infusionum* he describes the nucleus as giving off from its middle, at right angles to itself, a process, of a bud-like form, which presently becomes surrounded by a clearer ring of tissue derived from the substance of the parent being. Engelmann observed precisely the same thing take place in a large specimen of *Acineta quadripartita.*

The origin of the offspring of Acinetina by sexual reproduction is highly improbable, and has no support from observation. It is probably an act of internal gemmation. It is only in *Acineta mystacina, Urnula epistylidis,* and *Podophrya fixa* that the production of these internal germs has been unnoticed; and as far as our knowledge at present extends, these three species multiply only by simple fission. But even in other Acinetina the internal production of germs is not an act of embryonic reproduction, but simply one of fission.

A second variety of reproduction, however, does occur among the Acinetina, as first noticed by Claparède in *Acineta quadripartita* and *A. cucullulus.* This consists in the breaking-up of the nucleus into several spheroidal segments, from which a still larger number of small ciliated germs derive their origin, distinguishable in no respect from the embryos of *Epistylis plicatilis.* Claparède represents this offspring so derived as similar to the usual form of Acineta-germs: but with this view Engelmann cannot agree; for he maintains that both the mode of origin and the nature of the mature germs so derived differ altogether from those of the usual offspring of *Acinetæ.* Thus it is a circumstance of much weight that these little Acineta-embryos derive their substance entirely from the nucleus, and receive no addition from the parenchyma of the parent animalcule. Again, they exhibit this peculiarity in their formation in common with the embryos of ciliated Infusoria, as, for instance, with those of Vorticellina; and if their external resemblance to these latter be also considered, we must admit the existence of a remarkable similarity between the embryonic development of Ciliata and that form of the reproduction of *Acinetæ* by these remarkable little germs. This being granted, it has an important bearing upon Stein's celebrated hypothesis of Acinetiform development of Infusoria. To show, for example, that the little Acineta-products in question are true embryos developed by a sexual reproductive act, is to overturn the Acineta-theory. On the other hand, if, as is possible, and as the completely similar organization of the little offspring of *Acineta quadripartita* to the embryos of *Epistylis plicatilis* intimates, this method of development has a special character, and is concerned in a transformation of the *Acinetæ* back again into ciliated Infusoria, then the Acineta-theory becomes established. Future researches must decide this difficult problem.

As an appendix to his general remarks on the organization and reproduction of Infusoria, Engelmann appends a systematic history of several new genera and species he has discovered. The new genera named and described are *Chasmatostoma, Microthorax, Drepanostoma, Gastrostyla,* and *Astylozoon;* and the new species of previously described genera are *Lacrymaria elegans, Conchophthirus* (Pl. III. fig. 15) *curtus, Pleurotricha setifera, Uroleptus mobilis, U. agilis, Oxytricha strenua, O. parallela, Carchesium Aselli,* and *Epistylis nympharum.*

The new genera are thus severally characterized:—

CHASMATOSTOMA. Pl. III. fig. 16.

Figure constant, reniform, somewhat compressed, uniformly ciliated. A small oval oral aperture, having an undulating membrane affixed within it, occupies the middle of the flat ventral surface.

Chasmatostoma reniforme (Pl. III. fig. 16)

has probably been often confounded with *Colpoda cucullulus* and similar Ciliata. Anteriorly the reniform body rather tapers, but posteriorly it is obtusely rounded-off. A short œsophagus extends inwards and to the right side from the oval mouth, which is further furnished along its right side within its margin with an actively undulating fine membrane. Sometimes a second smaller membrane of the same kind is imperfectly seen on the left side. The nucleus is circular, placed in the median line in the posterior half of the body, and having a clearly defined round nucleolus occupying a hollow on its surface. The contractile vesicle is situated at the posterior extremity, and in its contraction acquires a stellate figure. It swims actively, mostly in a circuitous direction, by means of its uniform cilia, usually with its ventral surface downwards. Fission not observed. Size 0·06 millimètre.

MICROTHORAX. Pl. III. figs. 17, 18, 19.

Body loricated, compressed, oval, widely rounded posteriorly, uniformly ciliated. Mouth placed within the concavity of a rounded peristome situated in the left half of the body, and close to the posterior extremity. Nucleus and contractile sac single.

M. pusillus. Pl. III. fig. 17.

One of the smallest holotrichous Infusoria. It often occurs in large numbers together and in company with small Chilodons, with *Cyclidium glaucoma, Cinetochilum margaritaceum,* and *Pleurochilidium strigilatum,* and it is most closely allied to the two last-named animalcules. The left border of the rigid body is straight, whilst the right is convex. The peristome-depression is on the right side, and has an active valvular membrane within it. The right margin of the body is much thickened; and along the ventral aspect are two furrows, commencing from the anterior extremity, on the left side, and traceable to the middle of the body. The dorsal surface is not striated, but bears cilia. The round nucleus is central; the contractile vesicle near to and above the peristome, on its right side. Individuals colourless, their largest dimensions only 0·032 mill. Movements active; swims on the ventral aspect.

M. sulcatus. Pl. III. figs. 18, 19.

Much larger than the preceding, but of the same form. Along the dorsum are three deep longitudinal furrows; and the ventral sulci extend to the hinder border. The contractile vesicle is on the left side, and immediately above the peristome. Swims actively, on its ventral aspect. Size 0·057 mill.

DREPANOSTOMA.

Figure of body constant, but flexible, elongated, tapering gently in front and behind; ventral aspect flat, dorsal convex; the whole undersurface traversed by longitudinal striæ, with cilia of uniform length. The dorsum is smooth, but bears along each margin a row of bristly cilia. Mouth placed beneath a crescentic horny band not far from the anterior extremity, and close to the left margin of the body. Nuclei two, each with a nucleolus; no sharply circumscribed contractile space.

D. striatum,

the only species yet determined, is allied on the one side with hypotrichous Ciliata, such as *Chilodon,* and, on the other, with holotrichous *Amphilepti.* On account of the cilia being limited to the ventral surface, it must be placed among the hypotrichous Infusoria. The anal outlet is on the dorsal surface, not far from the posterior extremity. The body is from 4 to 5 times as long as broad, and somewhat pointed at its two ends. Fission not observed. Size from 0·185 to 0·25 mill.

GASTROSTYLA.

Figure constant; body loricated, elliptic, contracted anteriorly, and more or less obtusely rounded posteriorly. Five or six strong unciform setæ are situated on the anterior extremity, and a row of bristly cilia stretches obliquely across the ventral surface as far as the four or five strong cilia about the anal orifice, beyond which only a few extend to the posterior border. The two rows of marginal cilia increase in dimensions posteriorly, and overlap no setæ at the posterior extremity. Nuclei four.

G. Steinii

was noticed in January 1860, and belongs to the larger forms of Oxytrichina. It is two and a half times as long as broad. The frontal region is surmounted by a semilunar labium. The peristome is small in diameter; on its inner margin a strong undulating membrane is attached. The oblique row of setæ, stretching from the first adoral cilia in a gently curved line to the five or six strong cilia about the anal outlet, is a characteristic feature of the genus. Three stout uncinate setæ are inserted on the frontal region, and anteriorly to the oblique row of cilia are two other such setæ, and behind the angle of the mouth from one to three powerful cilia. A remarkable feature in this species, as in *Onychodromus,* is the existence of four nuclei, each with its nucleolus, situated in a line one behind the other. Movements rapid, not long in one direction. Fission proceeds as in *Stylonychia Mytilus.* The four nuclei coalesce into one large body, which by and by breaks up into eight segments, from each of which four germs proceed. The segments so subdividing generally present about their centre a fissure-like fold, recalling what happens in the nucleus of *Aspidisca, Euplotes,* and Oxytrichina under like circumstances. On the water around the *Gastrostyla* drying up, the animal readily encysts itself, the cysts resembling those of *Stylonychia Mytilus,* but rather more indented on their surface.

In company with *Gastrostyla* and *Cyclidium glaucoma,* some small circular *Acinetæ* were sometimes met with, resembling the embryos of *Stylonychia Mytilus,* and not improbably embryos of the Gastrostyle itself. This, however, is uncertain, as specimens of this animalcule were discovered with embryonic corpuscles. Size 0·15 to 0·32 mill.

ASTYLOZOON. Pl. III. fig. 20.

Body contractile, unstalked, campanulate, with an acutely pointed posterior extremity having a curve backwards. Surface smooth. Rotary organ extrusile, furnished with a spiral ciliary wreath. Peristome swollen, thickened. Posterior extremity terminated by one or two long locomotive setæ. Nucleus short, reniform.

A. fallax. Pl. III. fig. 20.

The only species known. At first sight, it looks like a detached *Vorticella microstoma,* but differs from it by its curved posterior pointed extremity, its rotary organ becoming wider behind obliquely, its small reniform nucleus, and its terminal locomotive setæ, whereby it can execute movements not effected by any detached *Vorticellæ.* It is frequently encysted. Size 0·1 mill. Size of cysts 0·038 to 0·05 mill.

EXPLANATION OF PLATE III.

Fig. 1. *Euplotes Charon,* in process of conjugation.

Fig. 2. The same animalcule at a later stage of conjugation: the new system of cilia is further developed, and the two beings are in course of separation.

Fig. 3. The globular vesicle developed internally has attained about its maximum growth.

Fig. 4. *Stylonychia pustulata,* in conjugation. The anterior extremities of the two animals connected by an intermediate band.

Fig. 5. The same, in conjugation according to a second variety of that process. The two beings, united most of their length, have begun the development of a new series of cilia.

Fig. 6. The same animalcules two hours later.

Fig. 7. One of the animalcules after the termination of conjugation and separation, twenty minutes after the stage shown in fig. 6.

Fig. 8. *Stylonychia histrio,* a peculiar phase of; the three globular vesicles subsequently coalesced into one large one, containing numerous granules.
Fig. 9. *Stylonychia Mytilus,* showing the formation of embryos and the development of germinal globules.
Fig. 10. *Urnula Epistylidis,* growing on the stem of *Epistylis plicatilis.*
Fig. 11. *Carchesium Aselli,* with a large embryonic vesicle: *a,* a detached embryo.
Fig. 12. Development of embryos within embryonic vesicles in the same animalcule.
Fig. 13. *Carchesium Aselli* strongly contracted, and with its ciliary disk elongated and circumscribed by a constriction, making it resemble an independent germ in course of growth.
Fig. 14. *Zoothamnium affine.* The nucleus has broken up into numerous minute globular corpuscles, collected within an outgrowth from its side, terminated by an aperture.
Fig. 15. *Conchophthirus Anodontæ* (Stein).
Fig. 16. *Chasmatostoma reniforme.*
Fig. 17. *Microthorax pusillus,* ventral aspect.
Fig. 18. *Microthorax sulcatus,* ventral aspect.
Fig. 19. The same, dorsal aspect.
Fig. 20. *Astylozoon fallax.*

XXV.—*Descriptions of a new Genus and some new Species of Naked Mollusca.* By JOSHUA ALDER and ALBANY HANCOCK, F.L.S.

THE following species of Nudibranchiate Mollusca have recently come under our notice, mostly through the kindness of our friends, to whom our thanks are due for the liberal manner in which they have communicated to us all specimens met with in this department of natural history that were likely to prove new or interesting.

Family **Dorididæ.**

Doris testudinaria, Risso.

Doris testudinaria, Risso, Hist. Nat. de l'Europe Mérid. vol. iv. p. 33, f. 15; Philippi, Enum. Moll. Sic. vol. ii. p. 78.

Body ovate or broadly elliptical, convex. *Cloak* large, of a chestnut-colour, with indistinct pale-yellowish blotches towards the sides, covered with smallish unequal tubercles, interspersed with a few larger ones, with minute pale lines radiating from them. *Dorsal tentacles* stout, yellowish, with fifteen to seventeen laminæ, divided in front by a groove: *oral tentacles* linear. *Branchial plumes* eight, tripinnate, forming an incomplete circle, open behind, retractile within a large cavity. Underside of the cloak orange- or lemon-coloured, spotted with reddish brown: